

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: ABATANGELO, G. et al

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Serial No. 09/445,604

JUN 27 2002

Filed : December 7, 1999

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Title: "Hyaluronic acid derivative based cell culture and biodegradable three-dimensional matrix."

DECLARATION UNDER CFR1.132

I, Davide BELLINI, do hereby declare as follows:

1. I am an Italian citizen residing at Montegrotto Terme (PD), Italy,
2. I am familiar with the English language.

I further declare that:

A) EDUCATION

In 1982 I received a diploma as Laboratory Technician from the *Istituto Statale "Duca d'Aosta"*, Este, Padua, Italy

B) PREVIOUS JOB EXPERIENCES

From 1984 to 1992 I worked as a laboratory technician in the 1st Research and Development Laboratory in the Chemical Research Department at Fidia Neurobiological Research Laboratories at Abano Terme, Padua, Italy, conducting research on hyaluronic acid, where I was involved in the following research projects:

- ☐ Organic synthesis starting from natural bases such as sphingosine, psychosine, dihydrosphingosine, relative to the project "Organic Amides";
- ☐ Research on natural polymers for devising methods to prepare ester derivatives of polysaccharides (hyaluronic acid, gellan, alginic acid).

Since 1992 I have been working as a technical expert in the Chemistry Laboratory at Fidia Advanced Biopolymers S.r.l. My current job includes research on natural polysaccharides such as hyaluronic acid and gellan. I work on the synthesis and chemical modification of said polymers to obtain new biomaterials. I develop prototypes of certain products (gels, films, membranes) for use in the various business areas (osteoarticular, tissue regeneration, anti-adhesion).



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I further declare as follows:

I am a co-author of the following abstracts

- 1) R. Toffanin, A. Flaibani, a. L. Segre, D. Bellini, A. Paparella "1H and 13C NMR Characterization of Auto-Crosslinked polymers of Hyaluronan", XXVI National Congress on Magnetic Resonance, Verona, September 1995.
- 2) Crescenzi V., Bellini D., Bertazzo E., Renier D., Imbiaco D.: "Chemistry of Hyaluronan (HA): Studies on the relationships between molecular structure, reactivity and physical properties of HA and derivatives". "Seminars on New Biomaterials", Brussels, December 1995.

and a co-author of the following papers:

- 1) Crescenzi V., Dentini M., Silvi F., Paci M., Paradossi G., Bellini D., Righetto Z.: "Case Studies of Physical and Chemical Gels based on Microbial Polysaccharides" Journal of Bioactive and Compatible Polymers (1994)
- 2) Mensitieri M., Ambrosio L., Nicolais L., O'Regan M., Bellini D.: "Viscoelastic properties modulation of novel autocrosslinked hyaluronic acid polymer". Journal of Materials Science: Materials in Medicine, Vol. 7, 695-698 (1996)
- 3) Crescenzi V., Francescangeli A., Renier D., Bellini D. "New cross-linked and sulfated derivatives of partially deacetylated hyaluronan: synthesis and preliminary characterization" Biopolymers Vol. 64, 86-94 (2002).
- 4) Crescenzi V., Francescangeli A., Renier D., Bellini D. "New hyaluronan chemical derivatives. Regioselectively C(6) oxidized products" Macromolecules Vol. 34, 6367-6372 (2001).

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I am first inventor of WO96/37519 and I am aware of the subject matter of the instant application.

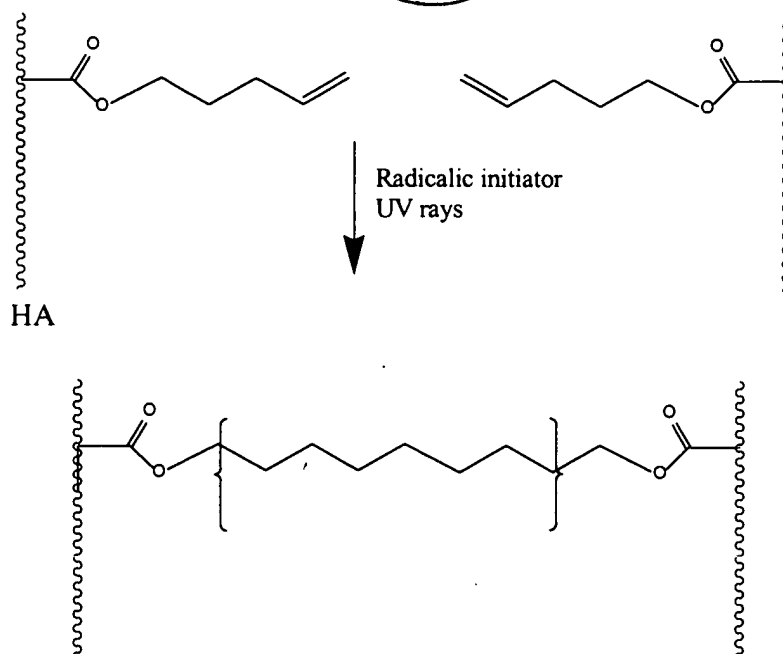
On the basis of my knowledge and my professional experience, I declare as follows:

The hydrogel material described in patent application WO 96/37519 is obtained by treating particular hyaluronic acid ester derivatives (HA) with different sources of energy such as UV light or gamma or beta rays in the presence of a radicalic initiator. Said ester derivatives are linear esters of HA obtained by reacting part of the carboxy groups of HA with alcohols belonging to the unsaturated series, that is, alcohols containing at least one C=C double bond.

In the presence of a radicalic initiator, the energy supplied by the UV light or by the gamma or beta rays causes the breaking of the double bonds present in the unsaturated alcohols that, by opening, form a carbon/carbon bridge with the double bond of the unsaturated alcohols of the next HA molecule, resulting in the cross-linking of several HA molecules, as shown in the following scheme:



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The formation of carbon/carbon bridges between HA molecules is therefore responsible for the molecular structure of the gel that is thus formed.

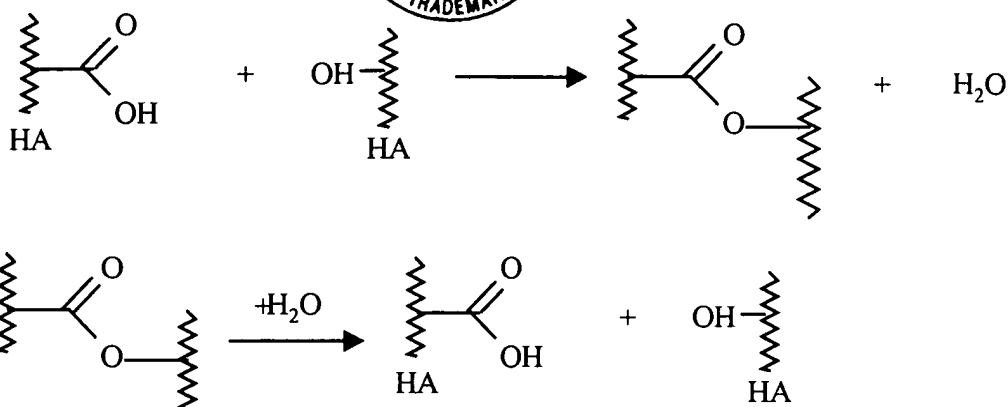
The hyaluronic acid derivatives of class A) of the instant application, which are described in EP 0216453, are linear hyaluronic acid molecules having some or all of the carboxy groups esterified with alcohols belonging to the aliphatic, aromatic, arylaliphatic, cycloaliphatic or heterocyclic series. In addition, among the derivatives of class A), those having carboxy groups esterified with unsaturated alcohols correspond to the intermediates used for the preparation of the hydrogel material of patent application WO 96/37519.

Thus, it is clear from the above that the polysaccharides of class A) have a completely different chemical structure from the polysaccharides of WO 96/37519, which also results in completely different physical properties.

The hyaluronic acid derivatives of class B) of the instant application are formed by the cross-linking of polymer chains of hyaluronic acid (HA) wherein part of the carboxy groups of the D-glucuronic residue react directly with hydroxy functions belonging to the same chain (intra-molecular bond) or to the next HA chain (inter-molecular bond), thus forming an ester-type bond. The chemical reaction that leads to the formation of this cross-linked product is a condensation process with loss of water molecules, as illustrated below:



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The chains are therefore cross-linked together directly without the presence of a "spacer" molecular bridge. This particular chemical structure results in special gelling properties even when the degree of cross-linking is actually very limited (5-10% of the carboxy groups involved in the reaction).

Also in this case, it is clear that the polysaccharides of class B) have completely different chemical structures and physical properties from the hydrogel material described in WO 96/37519.

I finally declare that all statements made herein are true of my own knowledge and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that such wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the applications or any patents or re-examination certificate issued thereon.

Date: May 15th, 2002

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